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UNIVERSITY OF ALBERTA

THE EFFECT OF PROLONGED THERMAL EXPOSURE ON RUMEN MOTILITY IN STEERS

BY

JACOB OLONGIDA OLE MIARON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

IN

ANIMAL PHYSIOLOGY

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

(SPRING 1990)

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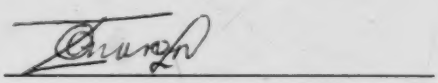

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THE UNDERSIGNED CERTIFY THAT THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS ENTITLED THE EFFECT OF PROLONGED THERMAL EXPOSURE ON RUMEN MOTILITY IN STEERS SUBMITTED BY JACOB OLONGIDA OLE MIARON IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ANIMAL PHYSIOLOGY.

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DATE April 11, 1990

ABSTRACT

Six 1-yr-old steers (1/3 Charolais x 1/3 Angus x 1/3 Galloway) fitted with permanent rumen cannulae and fed alfalfa-grass hay, were exposed to thermal treatments (-10, 10 and 28°C) to evaluate effects of prolonged thermal exposure on reticulo-rumen motility (frequency, duration and amplitude) and the consequential effect on apparent digestibility of dietary constituents such as dry matter, organic matter, neutral detergent fiber and crude protein. The passage rates of particulate matter and fluid from the rumen, metabolic heat production, plasma thyroid hormone concentrations, heart rate, respiratory rate and rectal temperature were determined.

The stress "indicators" i.e., heart rate, respiratory rate and rectal temperature were significantly ($P<0.05$) affected by the thermal treatment. The duration of reticular contraction during resting was significantly ($P<0.05$) lower at -10°C than at 10 and 28°C. The resting frequency of reticular contractions showed a quadratic response ($P<0.1$) to thermal exposure with the lowest value at 10°C. The changes in the apparent digestibility of dry matter, organic matter, neutral detergent fiber and crude protein were significant ($P<0.05$). The change observed was curvilinear with the lowest value at 10°C. The fractional outflow rate of ruminal particulate matter was unaffected by thermal exposure ($P>0.05$) but a quadratic response ($P<0.1$) of fluid outflow rate was noted with the highest value observed at 10°C. The effect of thermal exposure on

metabolic heat production was significant ($P<0.05$) and slightly curvilinear. Heat production at -10°C was highest compared to 10 and 28°C. Plasma triiodothyronine, but not thyroxine, concentration was significantly ($P<0.05$) higher at -10°C compared to 10 and 28°C. There was a significant negative correlation between the rumen fluid outflow rate and dietary constituent digestibility.

Prolonged thermal exposure appeared to have an influence on the frequency and duration of reticular contractions even when food intake is kept constant and the interaction of these reticulo-rumen motility indices with other factors such as fluid rate constant, which are believed to influence RR particulate and fluid dynamics is possible. Contrary to expectation, the apparent digestibility responses to prolonged thermal exposure, were non-linear with lowest values at 10°C, intermediate at -10°C and highest at 28°C.

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INTRODUCTION

When a mammal is exposed to a change in the thermal environment a variety of physiological adjustments occur (Hales 1974, Webster 1976, Thompson 1977, Alexander 1979, Sasaki et al. 1986). These adjustments range from minor circulatory and behavioural or postural to major neuroendocrine and metabolic adjustments. The magnitude of the response is modified by previous exposure to a thermal environment, the insulative properties of the fur, hair or wool cover and the level of feeding (Slee et al. 1982). Exposure to the so called "lower critical temperature" induces an immediate increase in metabolic rate to compensate for greater heat loss to the environment whereas exposure to high temperatures may result in lower metabolic heat production, usually associated with reduced feed intake (Webster 1976). These changes will enable the animal to cope better with unfavourable climatic conditions.

Prolonged exposure to a thermal environment results in acclimation to cold or heat involving changes in hormonal and sympathetic nervous activity. The endocrine involvement in the process of physiological adaptation to the thermal environment has been well documented (Yousef et al. 1965, Chaffee et al.1971, Sanchez 1972, Macfarlane et al. 1974, Thompson 1977) and involves a multiglandular response which is exerted mainly via the

hypothalamic-pituitary-adrenal axis with a major participatory role by the thyroid gland. Thyroxine and triiodothyronine are the major thyroid hormones and the latter is regarded as the active form of the hormone (Nicod 1976, Polk et al. 1989).

The digestive system is amongst the body systems i.e circulatory, respiratory and endocrine which show a response to thermal stress in ruminants. The low amplitude reticulo-rumen (RR) motility observed after vagotomy is intrinsically cholinergic, dependent upon the activity of the myenteric plexus, and is controlled by the degree of RR distension (Gregory 1982, 1984). The major contractions of the RR which constitute the 'primary' and 'secondary' contractions are controlled reflexly via the vagus nerve whose efferents originate from the gastric centers in the medulla oblongata (Iggo 1956). The gastric centers receive excitatory and inhibitory inputs from higher centres of the central nervous system, the digestive tract itself, and possibly from the peripheral warm and cold receptors of the skin. Conditions which have general effects on the central and peripheral nervous system or a change within the RR will affect motility. Likewise, any factors which affect intrinsic contractions will have consequential effects on extrinsic contraction and hence gut motility.

Stressors described according to their physical properties as thermal, chemical, electrical or mechanical are the main factors that affect the central and peripheral nervous system. Previous reports on digestive responses to thermal stress were not considered to have a physiological basis (Graham et al. 1959, Blaxter et al. 1961).

Studies at the University of Alberta on cold acclimated sheep and cattle (Christopherson 1976, Westra and Christopherson 1976, Kennedy and Milligan 1978) and elsewhere (Attebery and Johnson 1969, Warren et al. 1974, Nicolson et al. 1980) have established that the digestive response to the thermal environment is a physiological phenomenon. These responses include reduced dry matter and nitrogen digestibility, increased rumination (Kennedy et al. 1978, Kennedy 1985), increased frequency of reticular contractions (Westra and Christopherson 1976, Lirette et al.1988) in cold exposed sheep and cattle. Additionally, increased dry matter (Warren et al. 1974) and nitrogen (Mishra et al. 1970) digestibility and decreased frequency and amplitude of reticular contractions (Attebery and Johnson 1969) occur in heat stressed cattle. The effect of either acute or prolonged cold exposure on the duration and amplitude and of heat on duration of reticular contractions has not been documented.

Closely associated with temperature induced changes in motility are changes in the rate of passage of digesta through the digestive system (Warren et al. 1974) and rumen fluid turnover (Kennedy et al. 1976). Changes in motility are suggested to cause increases in physical propulsive movements of digesta from the reticulorumen of sheep exposed to cold stress (Westra and Christopherson 1976). The majority of studies involving the effect of thermal stress on the digestive system of ruminants have been done on sheep exposed to acute or prolonged thermal treatments. On the other hand, much of the literature on the effect of heat and cold on cattle describe experiments in which animals were taken from their natural

environment and exposed for a short period to an acute thermal stress. These types of experiments will not necessarily reflect the chronic effects of a particular environment on the digestive physiology of the ruminant animal. Furthermore, conclusions drawn from studies in sheep may not be true in cattle because the anatomy and motility patterns differ from that of sheep (Reid, 1963). Therefore, further research is needed to examine the effect of prolonged exposure to different thermal environments on rumen function in cattle.

LITERATURE REVIEW

Thermoregulatory Systemic Responses To Thermal Environment.

When the ambient temperature falls outside the bounds of the upper or lower critical temperature, animals attempt to maintain thermostability by increasing or reducing heat loss and thus triggering various behavioural or physiological responses (Webster 1976, Thompson 1977, Sasaki and Weekes 1986). These responses are better viewed as systemic adjustments and they have been well documented to include the cardiovascular adjustment (Young 1975, Westra and Christopherson 1976, Berbegier 1987, Lirette 1988), respiratory responses (Murray 1966, Webster 1973, Young 1975, Monstma et al. 1985), endocrine changes especially the thyroid involvement (Leblanc 1971, Jansky 1971, Sanchez et al. 1972, Miller et al 1974, More et al. 1977, Westra and Christopherson 1976) and effects on the digestive system (Christopherson 1976).

Domestic bovids respond to cold by increasing heart rate (Webster 1974b, Young 1975, Lirette 1988) and metabolic heat production (Young 1975, Christopherson 1976) and by decreasing respiratory rate (Webster 1974b) and respiratory evaporative heat loss. Exposure to heat results in elevated heart rate (Berbigier 1987), respiratory rate (Murray 1966, Bond et al. 1972, Webster 1973), cutaneous evaporative heat loss, rectal temperature (Murray 1966) and decrease in heat production (Webster 1974b). Heat stress depresses thyroid activity (Thompson 1975) whereas cold exposure increases thyroid function in cattle (Yousef et al. 1965, Christopherson et al. 1979). Studies in laboratory animals seem to favour a very minor participation of thyroxine in cold resistance (Leblanc 1971). It appears that triiodothyronine is more important in thermogenesis during cold stress situations (Leblanc 1971, Danforth et al. 1977). The basis for the increase T_3 activity in cold acclimated rats remains to be elucidated though one of several proposals is that cold stress could induce a rapid biotransformation of T_4 into the more active form. Nevertheless, administration of thyroxine increased metabolic rate and heart rate in cattle in a thermoneutral environment (Balch et al. 1952, Yousef and Johnson 1966).

Digestive Systemic Response To Thermal Environment.

The synchronized movements of the reticulo-rumen have been studied extensively (Schalk and Amadon 1928, Phillipson 1970, De Naville et al. 1987). The main function of one category of

reticulo-ruminal contractions, referred to as the 'primary cycles' (Schalk and Amadon 1928) or the "A" sequence contraction, is to promote mixing of digesta, fermentation, particle breakdown, absorption and onward passage of digesta. Additionally, secondary (B-Sequence) contractions occur in cattle and sheep (Stevens and Stellers 1959, Titchen and Reid 1965, De Naville et. al. 1987) in association with eructation of gas arising from rumen fermentation.

The effect of factors such as feeding and nature of meal or diet (Schalk and Amadon 1928, Dzuik et al. 1963, Titchen and Reid 1965, Colvin et al. 1978), rumination (Dziuk et. al. 1963, Kennedy 1985), fasting and dehydration (Nessic 1960, Attebery and Johnson 1969) and hormones (Grovum 1986) is well documented. However, information on the effect of thermal stress is scanty. When animals are exposed to varying environmental temperatures, numerous physiological adjustments occur, including changes in digestion even when food intake is held constant (Christopherson 1976). The physiological responses experienced by the digestive system depends on the nature of the thermal stress applied.

In cows exposed to acute heat stress at 38°C, the amplitude and frequency of reticular contractions were reduced, but not significantly. A five day exposure to 38°C resulted in a marked reduction in rumen contraction frequency and force of contraction as compared to that recorded at 18°C (Attebery and Johnson 1969). There was no indication of any particular threshold ambient temperature at which a rise in body temperature was sufficient to depress rumen contractility. Further investigation utilizing more than two

temperatures and periods longer than five days is needed to ascertain whether or not there is indeed a threshold temperature for influences on the digestive system.

In a thermoneutral environment, movement of digesta through the reticulo-omasal orifice may be affected amongst other factors by the frequency and amplitude of contraction of the RR (Kennedy et al. 1988) modulated by degree of tactile stimulation which depends on the physical properties of the diet (Faichney 1986). In most studies that relate particle passage rates to the RR motility, small particles (ca. 2 mm) have been used to estimate passage rates. Small particles associate with the rumen fluid phase (Bull et al. 1979), and therefore, may not be representative of all the rumen particulate digesta. Consequently, interpretation of data obtained from such experiments is complicated by the fact that in most cases the animal's actual feed is heterogenous, containing mostly larger particles with medium and small particles constituting a relatively small proportion. Other experiments, conducted under thermoneutral environments have shown that feed type and level may influence RR motility and the quantity of outflow per reticular contraction in sheep (Malbert et al. 1989). From their study Malbert and Baumont (1989) showed that sheep fed lucerne hay had higher "A" sequence contractions during eating and ruminating but not during resting and increased dry matter (DM) leaving the rumen per contraction when compared to sheep fed orchardgrass. This suggests the involvement of the "A" sequence contractions in the regulation of the RR digesta passage rate contrary to the observations of Freer et al. (1965) in

cattle and Ulyatt et al. (1984) in sheep. The latter two studies indicate that the amount of DM leaving the RR in a thermoneutral environment is not controlled by the frequency of A sequence contractions. Kennedy (1985) reported a decrease in the amount of DM outflow from the RR per reticular contraction in cold compared to warm acclimated sheep. Apart from frequency of RR contractions other factors may be involved in the control of RR particulate and fluid passage rates in ruminants exposed to acute or chronic thermal environment. One of the many possible factors is the fluidity and viscosity of the stomach content which is known to influence gastric emptying in dogs (Russel et al. 1985) and in sheep (Malbert et al. 1989). In many studies, the influence of water consumption on particle and fluid turnover rates has been overlooked.

Westra and Christopherson (1976) suggested that the rate of passage of digesta through the gut is influenced by changes in RR motility induced by thermal stress. Faster passage of dietary residues through the gastrointestinal tract is apparently responsible for depressed digestibility in cold-exposed sheep (Westra and Christopherson 1976). Further, studies at the University of Alberta have shown that although the frequency of reticular contractions increased in a cold environment, digesta flow per contraction tended to decrease (Kennedy 1985). This is rather unexpected and further study in sheep and cattle to investigate the relationship between temperature-induced motility, rate of contractions, force of contractions and duration of contractions is required. Steers exposed to high ambient temperatures of 32°C had a longer mean retention time

of digesta in the whole tract and thus increased dry matter digestibility (Warren et al. 1974). From the experiments of Warren et al. (1974), it is not clear whether increased retention time with high ambient temperatures is a common phenomenon for all the compartments of the digestive tract. Further studies will be required to provide this information.

Thermal stress is also known to influence fluid dynamics in the rumen. It has been reported that rumen fluid volume decreased during cold exposure in sheep (Kennedy et al. 1976, Weston 1977, Kennedy and Milligan 1978). Reduced retention time of particulate digesta markers in the rumen is associated with rapid turnover of fluid markers during cold exposure (Kennedy et al. 1976, Kennedy et al. 1978), and decreased rumen volume was coupled with an increased dilution rate of the fluid marker Cr-EDTA with no change in the volume of fluid flowing out of the rumen per day (Kennedy et al. 1978). While increased rumino-reticulum motility may account for the increase in turnover rates of particulate matter in the rumen, it is not clear what contributes to the decrease in rumen fluid volume of sheep during cold exposure.

Although, the actual mechanism inducing changes in the RR contractions and consequently, digesta passage rates in GIT of domestic ruminants exposed to different thermal environment are as yet to be elucidated, neural and endocrine systems have been implicated (Christopherson 1985). In a thermoneutral environment Gregory (1982, 1984) working with anaesthesized sheep, suggested that the low amplitude RR motility depends upon the activity of the

myenteric plexus. Iggo (1956) showed that, in goats, the major contractions constituting the "primary" and "secondary" contractions of the RR are controlled by the vagus efferents originating from the gastric centre(s) of the medulla oblongata. This along with the sympathetic nerve supply via the splanchnic nerves, constitutes the extrinsic neural supply to the gut. Other studies (Szurszewski 1987, Weyns et al. 1987) show that the GIT of most mammals including domestic ruminants is richly innervated by complex and interconnected intrinsic neuronal networks which constitute the so-called "enteric nervous system" (ENS).

The ENS is characterized by the presence of putative and established neurotransmitters and peptides (Weyns et al. 1987) structurally similar to those found in the brain. The magnitude of smooth muscle intrinsic contractions may reflexly influence the rate and amplitude of the extrinsic contractions and that putative neurotransmitters such as vasoactive intestinal polypeptide (VIP), enkephalins, substance P, etc. may take part in modulating this response. At present, it is not possible to define the exact nature of the interaction between the environmental temperatures and the GIT. However, it is suggested that it may be mediated via the endocrine, central and enteric nervous systems. Evidence from a review (Oliviero 1987) indicates that both the hypothalamic-pituitary-adrenocortical (HPA) and sympathetico-adreno-medullary (SA) systems are activated or inhibited by exposure to stressful conditions. Recent immunohistological studies (Kitamura et al. 1986, 1987) indicate that, the RR, reticular groove,

reticulo-omasal orifice (ROO) and the omasum contain nerves with abundant peptidergic immunoreactivity. Most of the peptides demonstrated include VIP, substance P, leucine enkephalin and gastrin releasing polypeptide. These peptides together with factors that might influence the cholinergic and non-cholinergic receptors known to inhabit the walls of the ruminant forestomachs may hold the key to our understanding of thermally-induced changes in the RR contractions. In fact, VIP has been shown to evoke a complete relaxation of the lower esophageal sphincter in dogs (Gonda et al. 1989) as well as inducing a relaxation of ROO in lambs (Reid et al. 1984) suggesting a possible role in the control of the digesta passage rate. Further research investigating the effect of thermal exposure on the modulatory role of these neuropeptides on the motor activity of the ruminant forestomach is highly recommended.

Water Consumption and Thermal Stress.

Water turnover in domestic bovids is affected by thermal exposure (Degen and Young 1980) and is lower in winter than in summer (Siebert et al. 1969, Hoffman et al. 1972). Although moisture loss in cattle kept in a thermoneutral environment is relatively low (Brody 1949), when drinking water is cooler than the air temperature, animals increase their water consumption with increasing environmental temperatures. Consequently, with rising ambient temperatures the concentration of blood constituents tends to decrease provided cool drinking water is available. Experiments with laboratory animals indicate that exposure to heat stress results in

increases in plasma volume, blood volume and dilution of plasma (Horowitz et al. 1989) and water deprivation causes a significant reduction in plasma volume and suggest that at higher temperatures water intake or turnover is highest and that water deprivation influences intra and extra-vascular fluid parameters. There is free movement of water between compartments as shown by the work of Bianca (1970) who reported that in dehydrated cows all blood parameters that have been affected by restricted water intake were restored to normal by rehydration, although there was a conspicuous difference in the pattern of restoration according to whether rehydration occurred by drinking or by infusion into the rumen. Redistribution of body water among the different compartments, including the rumen is therefore, possible in response to temperature.

Generally, research dealing with the interrelationships between the varied factors that might directly or indirectly influence particle and fluid turnover rates in ruminants and hence their productive performance, have not been systematically conducted. Accordingly, information on the effect of prolonged thermal exposure on one of the primary factors suggested i.e. RR motility in cattle is sketchy and inconsistent partly because, in most cases, only two temperatures and short periods have been studied. The major objectives of the study presented in this thesis are to examine the effect of prolonged thermal exposure on the form and pattern of reticular contractions, rumen particle and fluid turnover rates, apparent digestibility of dietary constituents, water consumption and metabolic heat production. For comparison several cardiovascular, respiratory and endocrine systemic responses were also investigated.

MATERIALS AND METHODS

ANIMALS, MANAGEMENT AND EXPERIMENTAL DESIGN

The experimental animals were six 1-yr-old steers (1/3 Angus x 1/3 Charolais x 1/3 Galloway) locally known as a the Pee-Wee strain, selected for small weights and bred at the University of Alberta ranch. The steers weighing 282-360 kg, were fitted with rumen cannulae (10 cm diameter). The animals were given a minimum of 3 weeks to recover from surgery and then were transferred into individual metabolic crates where they underwent acclimation for digestion and metabolic experiments.

Three temperature treatments (-10, +10 and +28°C) were applied to each experimental group consisting of two animals per group in a latin square design. Our periods were a chronological treatment sequence. The steers were grouped according to their live weights.

A diet consisting of alfalfa grass hay was offered at a rate calculated to meet 1.3x the maintenance requirement of steers (Table 1). The calculation was based on the National Academy of Science guidelines (National Research Council 1984). All animals were fed by means of an automatic feeding device which delivered the daily ration in 12 equal portions at intervals of 2 h. Water and cobalt-iodized salt were available ad libitum.

MEASUREMENTS

MOTILITY

Motility of the reticulum and the ventral sac of the rumen were monitored using open-ended catheters inserted into each region and kept in position by attached weights. A continuous slow infusion of water with a pump (Ismatec MT-13) kept the open tip of the catheters free of ingesta particles. The other end of the catheters were connected to pressure transducers (Gould Statham) which in turn were connected to a Beckmen recorder (Model R511A). To prevent freezing of the water in the catheters when measurements were made at -10°C the method described by Hills et al. (1977) was modified such that instead of hot water, hot air was circulated through a larger tube which surrounded the catheters using a gas pump (General Electric A-C Motor Fort Wayne, Indiana). Measurements were made on two animals simultaneously on each of the last three days of each 21 d period. The recordings started at 0800 h and were done continuously for 3 h and included at least one feeding time. Duration and amplitude of reticular contractions were estimated from peak width and heights respectively on the pressure recording (Appendix Figure 4). The pressure measuring system was calibrated with mercury and water manometers.

BLOOD SAMPLING AND ANALYSIS

Blood (20 ml.) was collected at 1000 h using a heparinized veni- puncture (Vacutainer, Becton Dickinson, Mississauga, Ont.)

after 21 d of thermal exposure. The blood was immediately centrifuged at 3000rpm for 15 minutes and plasma was stored at -20°C until analysis for triiodothyronine (T_3) and thyroxine (T_4). The total T_3 and T_4 concentration was measured using commercial radioimmunoassay kits (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA).

HEART RATE

Heart rate (HR) was recorded by ausculation with the aid of a stethoscope. Heart rate was recorded at 0700 h every day throughout the experimental period but values for the last week of each of the three 21 d periods were used for statistical analysis.

RESPIRATORY RATE

Flank movements were counted once daily at 0700 h to determine respiratory frequencies. Values from the last week of each of the three 21 d period were used for treatment comparisons.

RECTAL TEMPERATURE

A telethermometer (Model 46 TUC YSI) was used to measure rectal temperature. The probe was inserted 15 cm into the rectum and recording was done between 0700-0800 h throughout the experimental period, But only values from the last week of each of the three 21 d period were used for treatment comparisons.

METABOLIC RATE

The metabolic rate (MR) was measured using an open-circuit respiratory apparatus (Young et al. 1975) connected to a ventilated face mask to which steers had been previously accustomed. Ventilation rate of the mask was read from a flowmeter (Rotometer, Fisher and Porter, Warminster, Pa) and oxygen concentration difference between the incoming and outgoing air, from a single channel paramagnetic oxygen analyzer (Servomex 540A Sussex, England). The measuring system was calibrated with nitrogen as zero gas and by the procedure of Young et al. (1984). Heat production was calculated using the equation of McLean (1972).

MARKER PREPARATION

Chromium-mordanted fiber was used as a particulate marker. The fiber for mordanting was put through a two and a half wash with extra rinse cycle in an automatic washing machine. The fiber was then soaked in acetone for 24 h, washed, dried at 100°C and mordanted according to the procedure of Uden et al. (1980). The fluid passage rate was determined by crystalline CoEDTA, the crystals were prepared according to the method outlined by Uden et al. (1980).

MARKER ADMINISTRATION AND SAMPLING

The chromium (Cr) content of the alfalfa grass hay labeled in the present experiment was similar to the lowest Cr content used by Ehle et al. (1984) so little effect on density and passage would be expected. The mordanted fiber used in this experiment contained about

25mg Cr/g fiber comparable with particles used by Ehle et al. (1984), Robles et al. (1981) and Pond et al. (1989). Digesta passage rate determination was done from the 16th day of each of the three 21 d periods. A pulse dose was introduced into the rumen via a fistula at 0600 h. In this procedure about 1 kg of digesta was removed from the rumen, mixed thoroughly with Cr-mordanted fiber and returned into the rumen. The amount of Cr-mordanted fiber given was calculated to equal 2 g of pure Cr. After dosing, fecal and ruminal grab sample collections were initiated at 6 h and 10 h for rumen and fecal respectively and thereafter at scheduled intervals for 4 d. Each sample consisted of six subsamples from the cranial, medial, caudal and dorsal, medial and ventral regions of the RR. The subsamples were mixed thoroughly and a final sample was taken and frozen at -10°C until laboratory analysis.

Crystalline CoEDTA was administered as a pulse dose of 16g. The bolus was introduced intraruminally at 0700 h on day 21 of each of the three 21 d periods. Rumen fluid samples were collected initially at 0.5 h intervals for the first hour and thereafter at scheduled 1 h intervals for the next 9 h. Rapid collection was achieved by the use of a modified rumen fluid sampling device first developed by Bjornhag et al. (1984). The samples were frozen at -10°C until further analysis.

DIGESTIBILITY STUDY

Samples of diet were collected daily. The daily samples were composited for each digestibility trial. The daily fecal outputs were

collected once every 24 h at about the same time each day. The feces were collected in plastic trays placed on the floor beneath the elevated metabolic crates, weighed and an aliquot of 5% of each collection was stored at -10°C until the end of the collection period. The daily samples from each steer were than thawed, combined and mixed thoroughly to form a composite sample from which a final representative sample was obtained.

LABORATORY ANALYSIS

All the samples for the analysis were dried in a force-draft oven at 80°C and ground to pass through a 1 mm screen.

Particulate Passage Rate

Rumen or fecal samples (300-500 mg) were digested with 4M nitric acid (Murthy 1971) for 4 h at room temperature and then for 24 h at 75°C. The diluent was filtered through a #54 Whatman paper (Whatman, Clifton, NJ) into 10 ml test tubes. The clear solution was analyzed for Cr by atomic absorption spectrophotometry (AAS) (Model 4000, Perkin Elmer Corp., Norwalk Conn. 06856, USA) according to standard methods (Williams et al. 1962, Binnert et al. 1968). A rumen and fecal sample was taken prior to dosing for background marker analysis.

Rumen Fluid Dilution Rate

Samples of rumen fluid were thawed, shaken and filtered through the #54 whatman filter paper and the Co concentration was determined

after a 1:10 dilution with 1M-hydrochloric acid by AAS

Digestibility

The DM of feed and fecal samples were determined by oven drying at 80°C to constant weight. OM was determined on dried samples by ignition in a combustion furnace at 550°C overnight. NDF was analyzed according to the method of Goering and Van Soest (1970). N concentration of feed and feces was determined by the Macro-Kjeldahl method (Association of Official Analytical Chemists 1975).

CALCULATIONS

Particulate and Fluid Rate Constants

Linear regression analysis was used to measure slopes of the declining phase of the marker disappearance or dilution curves (Appendix Figure 2 and 3). Natural logarithm of the extracted ruminal and fecal chromium concentrations were correlated with the standard curve generated from pure Cr Standards. Calculation for the Co concentration were done according to procedure outlined for Cr.

Rumen Dry Matter Content And Volume

Initial rumen dry matter content was estimated from equation 1.

Pool Size= Dcr/C_0 1

Where Dcr = dosed chromium in (mg)

C_0 = concentration of chromium at time zero, this value was obtained by extrapolation of the regression line of the Cr

disappearance curve to time zero. Similarly, initial rumen fluid volumes were calculated from the dose of CoEDTA (mg) and the concentration (mgL^{-1}) of the marker at time zero.

The disappearance of digesta and fluid from the rumen was calculated with reference to Cr-mordanted fiber and CoEDTA respectively. The alfalfa-grass hay fiber was mordanted as fed. The Cr mordanted fiber consisted of 98% long particles. Medium and small particles constituted the balance as determined by wet sieving. Long particles were those that could not pass through a 4 mm screen while, medium and small particles could not pass through 2 and 1 mm screens respectively. Correction for absorption of Co across the rumen wall was not done because the urine Co concentration was below the detection limit of the AAS apparatus used for analysis. Similar observations were reported by Okine et al. (1989a) working in the same laboratory.

Apparent Digestibility Coefficients

Apparent DM digestibility coefficients were calculated using equation 2.

% Apparent digestibility = [(Feed DMg - Fecal DMg)/Feed DMg]*100....2

Apparent digestibility for OM, NDF and N were calculated by substituting the appropriate intake and excretion values in equation 2.

Water Consumption

Water consumption was determined daily from the residue left in

a 60 kg reservoir in the warm temperature treatment. In the cold, water containers were fitted with heating elements to prevent the drinking water from freezing and water intake measurement was done twice daily. The animals received fresh drinking water every day. Water intake was determined for 5 d during the steady state i.e from the 15^{th} d of each of the three 21 d periods.

STATISTICAL ANALYSIS

The data was analyzed by least square ANOVA utilizing the GLM procedure of SAS (1985). The model included the main effects, animal and period. Significant LS means were tested by Student's Newman Keuls multiple range test (Steel and Torie 1980).

RESULTS

All results were computed from data collected during the steady state of each of the three 21 d periods. The steady state was considered to be the period between 14th and 21st d of each of the three 21 d periods.

Feed Intake

There were no feed refusals and the controlled level of intake was similar for all temperature treatments (Table 2).

Physiological Responses

The effect of prolonged thermal exposure on several physiological parameters is shown in Table 3. The effect of thermal exposure was significant ($P<0.05$) in all but one case. There was a significant increase in heart rate (HR) in steers acclimated to both -10°C and 28°C compared to those at 10°C. Respiratory frequency (RR) was significantly ($P<0.05$) greater at 28°C compared to values during exposure to 10 and -10°C. There was a slight but statistically significant decrease in rectal temperature (Tr) in steers exposed to -10°C and a significant increase at 28°C compared to 10°C. The total plasma concentration of triiodothyronine T_3 (Figure 1), but not

thyroxine T_4, was elevated ($P<0.05$) in steers exposed to -10°C.

Motility

Table 4 shows the effect of prolonged thermal stress on the frequency, duration and amplitude of reticular contractions. The duration of reticular contractions during resting was significantly reduced during prolonged cold (-10°C) exposure ($P<0.05$) and the frequency of reticular contractions at rest showed a quadratic response with highest frequencies at -10 and 28°C. The amplitude during resting was not affected by prolonged thermal exposure. During rumination and feeding the frequency, duration and amplitude were not affected by prolonged thermal exposure.

The frequency of rumen contractions was not significantly affected by temperature (Table 5) but during rumination and feeding, the reticular and ruminal frequency of contractions was elevated in most cases compared to the resting state.

There was a significant ($P<0.02$) correlation ($r^2 = 0.53$) between heart rate and the frequency of reticular contractions during resting.

Heat Production

In Table 6 metabolic heat production (HP) has been expressed in watts (W) and ($Wkg^{-3/4}$) to adjust for any differences in body weight. HP was altered significantly ($P<0.05$) by thermal exposure. HP was highest at -10°C as compared to that measured at a warmer 10°C or higher 28°C temperatures. When measured in $Wkg^{-3/4}$, cold stress

values were almost twice the heat stress values and the inverse relationship between temperature and HP was slightly curvilinear (Figure. 2).

Digestibility

It appears that there is a direct relationship between dietary constituent digestibilities and thermal stress exposure (Table 7). The DM digestibility was lowest and significantly different ($P<0.05$) at a medium temperature of 10°C than at the colder (-10°C) and higher (28°C) temperatures. In addition, digestibility was slightly but not significantly lower at -10°C compared to 28°C. The changes in OM, NDF and CP digestibility in response to thermal exposure were similar to changes in DM digestibility.

Particulate passage rate constant

The particulate rate constants (PRC) (Table 8) were not significantly affected ($P>0.05$) by temperature. However, the PRC was slightly higher in the cold-exposed steers compared to steers held at 10°C and 28°C. The rumen dry matter content (RDMC) and particulate passage rate (PPR), calculated from the PRC were not affected by thermal exposure (Table 8). The PRCs estimated from the rumen and fecal samples were similar (Appendix Table 1).

Rumen Fluid Rate Constant (FRC)

Table 9 shows the effect of prolonged thermal exposure on the rumen fluid kinetics. There was a quadratic response ($P<0.1$) of the FRC with the temperature treatments which approached significance. The FRC was highest at 10°C and lowest at 28°C. Values at -10°C were intermediate. Rumen fluid volumes (RV) were similar at -10 and 10°C but slightly lower at 28°C and fluid outflow rates (FOR) showed a similar trend as the FRCs.

Water Consumption

Water intake was lowest at -10°C, compared to that at 10°C and 28°C (Table 9).

The significant changes in ($P<0.05$) dietary constituent digestibilities were associated with higher FRCs and FORs at a medium temperature 10°C and intermediate and lower values at -10 and 28°C respectively (Tables 7, 9).

There were negative correlations between rumen fluid outflow rates and DM, OM, NDF and CP digestibility $r^2 = 0.57$ $P<0.03$; $r^2 = 0.57$ $P<0.03$; $r^2 = 0.50$ $P< 0.06$ and $r^2 = 0.46$ $P<0.08$ respectively when all the treatment effects were pooled. Although, the R^2 values were not very high the correlations were significant for the digestibility traits measured and the levels of significance based on t-statistics were within $P<0.08$ and $P<0.03$.

Table 1. Composition of alfalfa-grass hay ration.

Dry matter (%)	93.28
Organic matter (%DM)	93.26
Neutral detergent fiber (%DM)	59.62
Crude protein (%DM)	13.75

Table 2. Feed consumption of steers exposed to -10°C, 10°C and 28°C (LSmeans±S.E.M[#]).

	Environmental temperature (°C)			
	-10	10	28	S.E.M
Daily dry matter (DM) intake				
kg	8.95	8.82	8.95	0.18
%body-wt	2.65	2.65	2.63	0.02

[#] S.E.M of treatment means (n 6).

Table 3. The effect of prolonged thermal environment on heart rate HR (beats min^{-1}), respiratory rate RR (breaths min^{-1}), rectal temperature Tr (°C), plasma triiodothyronine T_3 (ng $100ml^{-1}$) and thyroxine T_4 (ng ml^{-1}) concentration. (LSmeans±S.E.M[#])

	Environmental Temperature (°C)			
	-10	10	28	S.E.M
HR	75.5^{a}	65.9^{b}	74.6^{a}	2.1
RR	17.4^{a}	23.3^{a}	67.9^{b}	3.7
Tr	38.1^{a}	38.4^{b}	38.8^{c}	0.1
T3	185.0^{a}	126.3^{b}	127.3^{b}	9.9^{f}
T4	65.9	58.3	61.1	2.6^{f}

^{a,b}Within horizontal rows LSmeans with different superscript letters were significantly different ($P<0.05$)

[#]S.E.M of treatment means (n 6)

^{f}S.E.M of treatment means (n 5)

Table 4. The effects of prolonged thermal environment on reticular contractions. (Lsmeans±S.E.M#).

	Environmental temperature (°C)			
	-10	10	28	S.E.M
Reticulum Motility				
Frequency (Contractions Min^{-1})				
Resting*	1.26	1.08	1.20	0.07
Ruminating	1.26	1.16	1.35	0.07
Feeding	1.72	1.58	1.65	0.16
Duration (seconds)				
Resting	5.35^{b}	5.67^{a}	5.73^{a}	0.09
Ruminating	9.30	9.35	9.41	0.23
Feeding	5.30	5.59	5.58	0.12
Amplitude (mm Hg)				
Resting	5.18	4.62	5.15	0.58
Ruminating	4.67	4.08	4.20	0.52
Feeding	4.58	5.24	4.59	0.71

^{a,b}Within horizontal rows LSmeans with different superscript letters were significantly different ($P<0.05$).

*Quadratic response with resting frequency of reticular contractions ($P<0.1$)

#S.E.M of treatment means (n 6).

Table 5. Effect of prolonged thermal environment on frequency of reticular and ruminal contractions (contractions h^{-1}).
(LSmean±S.E.M[#])

	Environmental temperature (°C)			
	-10	10	28	S.E.M
Reticular				
Resting *	76	65	72	3.90
Ruminating	75	70	81	4.00
Feeding	103	95	99	9.45
Ruminal				
Resting	114	95	97	10.55
Ruminating	123	110	106	24.42
Feeding	115	129	129	7.62

[#]S.E.M of treatment LSmeans (n 6).

*Quadratic response with resting frequency of reticular contractions ($P<0.1$)

Table 6. The effect of prolonged thermal environment on metabolic heat production of steers. (LSmean±S.E.M#)

	Environmental temperature (°C)			
	-10	10	28	S.E.M
Heat production				
W	636.03[a]	489.17[b]	394.63[c]	17.11
$Wkg^{-3/4}$	8.24[a]	6.20[b]	4.90[c]	0.20

a,b,c within horizontal rows LSmeans with different superscript letters were significantly different ($P<0.05$).

#S.E.M of treatment LSmeans (n 6)

Table 7. The effect of prolonged thermal environment on total dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and crude protein (CP) apparent digestibility. (LSmeans±S.E.M#)

	Environmental temperature (°C)			
	-10	10	28	S.E.M
DM (%)	72.19^{ab}	68.49^{b}	76.42^{a}	1.30
OM (%)	76.43^{ab}	73.41^{b}	79.79^{a}	1.12
NDF (%)	69.52^{ab}	64.58^{b}	73.86^{a}	1.55
CP (%)	75.38^{ab}	72.77^{b}	78.29^{a}	1.14

^{a,b} Within horizontal rows LSmeans with different superscript letters were significantly different ($P<0.05$)

#S.E.M of treatment means (n 6)

Table 8. Particulate rate constant(PRC), rumen dry matter content (RDMC) and particulate passage rates (PPR) of steers exposed to prolonged thermal treatment. (LSmeans±S.E.M[#])

	Thermal treatment (°C)			
	-10	10	28	S.E.M
PRC(h^{-1})	0.042	0.039	0.033	0.003
RDMC(kg)	3.02	3.09	3.68	0.44
PPR(kg h^{-1})	0.123	0.116	0.117	0.02

[#]S.E.M of treatment means (n 6)

Table 9. Rumen fluid rate constant (FRC), rumen volume (RV), rumen fluid outflow rates (FOR) and water intake (WI) of steers exposed to prolonged thermal treatment. (LSmeans±S.E.M[#]).

	Thermal treatment (°C)			
	-10	10	28	S.E.M
FRC(h^{-1})*	0.183	0.225	0.142	0.030
RV(L)	40.89	41.02	36.75	7.41
FOR(Lh^{-1})*	7.48	9.23	5.22	1.03
WI(Ld^{-1})	22.03[a]	30.77[b]	33.98[b]	2.20[f]

[a,b]Within horizontal rows LSmeans with different superscript letters were significantly different ($P<0.05$)

*Quadratic response with rumen fluid dilution rate constant ($P<0.1$)

[#]S.E.M of treatment means (n 5).

[f]S.E.M of treatment means (n 6).

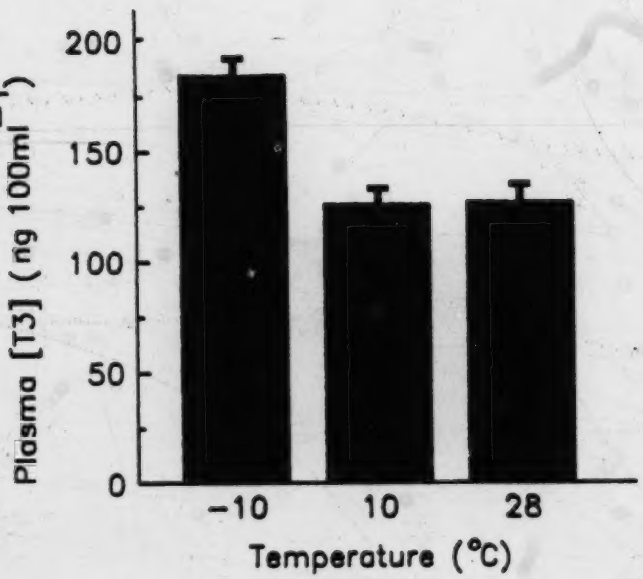

Figure 1. Triiodothyronine plasma concentration of steers exposed to prolonged thermal treatment

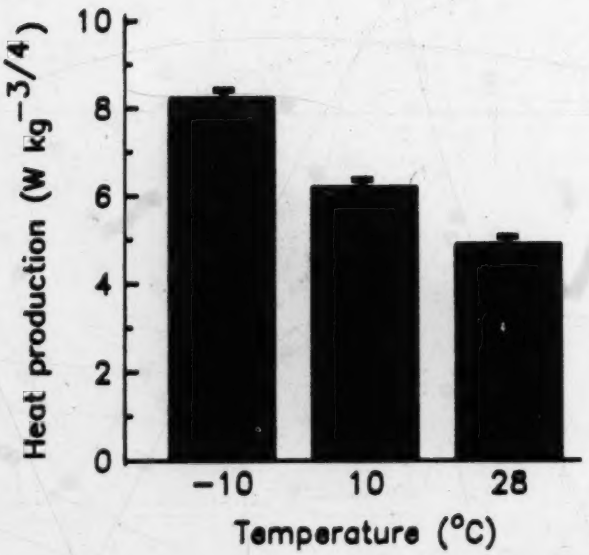

Figure 2. Effect of prolonged thermal exposure on metabolic heat production of steers

DISCUSSION

There was a similar level of food intake in all environments since the steers were given a fixed intake. This feeding strategy was planned so as to avoid confounding effects of differences in feeding level. However, interpretation of the results will need to take account of differences in water intake since these might affect the digestive responses.

The increased heart rate in both cold and heat exposed steers is indicative of stress. The elevation of heart rate in cold acclimated steers is in agreement with the findings of Lirette et al. (1988). Ruminants in the cold environment show increased sympatho-adrenal activity (Graham et al. 1981) which via catecholamine secretion might influence heart rate. The influence of catecholamines on cardiac functions could be potentiated by the thyroid hormones. Numerous studies on experimental animals in vivo and in vitro, have addressed the effect of thyroid hormones on the sensitivity of the heart to catecholamines. In some studies, thyroid hormones enhanced the stimulatory effects of catecholamines on cardiac functions, while in others the effect of thyroid status on cardiac sensitivity to catecholamines remain obscure (Skelton et al. 1986). It is possible that the complexity of the interaction between thyroid hormones and

catecholamines is compounded by the fact that thyroid hormones exert direct effects on the heart independent of catecholamines (Thier et al. 1962, Wildenthal 1971, Rutherford et al. 1979) and that thyroid hormones potentiate cardiac responses to agents other than catecholamines (Coville et al. 1970). Nonetheless, these studies provide very modest support for the hypothesis that sensitivity to cardiac effects of catecholamines is enhanced by thyroid hormones although appreciable ambiguity still exists.

Exposure to 28°C imposes a thermoregulatory requirement to reduce the impact of the excessive heat load. This is manifested by an increase in heart rate with a subsequent increase in blood flow to the periphery, where heat is lost to the environment. This is consistent with the observation that cattle in the sun (analogous to high temperature in this study) had higher heart rates than those in the shade (Berbiger 1987).

The respiratory response in this study is consistent with the work of other researchers (Bond et al. 1972, Young et al. 1975, Gonyou and Christopherson 1979, Monstma 1985, Mcguire et al. 1989). Respiratory rate is indicative of the thermoregulatory status of the animal with the lowest rate occurring when the animal is exposed to temperatures below its lower critical temperature (Webster 1973). The respiratory response was linear with the lowest frequency at -10°C and highest at 28°C. The reduced respiratory frequency at -10°C is suggestive of the reduced loss of heat due to evaporation from the respiratory tract at low temperatures, while elevated rates of respiration at 28°C indicates the existence of a heat stress

situation and an increase in evaporative heat loss. Similar patterns of respiration frequency have been observed in cows (Young 1975), in heifers (Webster et al. 1970) and in steers (Gonyou et al. 1979).

The thermogenic activity of catecholamines in the cold could be potentiated by elevated thyroid hormones (Roy et al. 1977, Fregly et al. 1979, Fain 1981). In the present study, prolonged cold exposure induced an increase in the more biologically active T_3 but not T_4. These results differ only slightly from those of Christopherson and Thompson (1983) who reported that cattle and sheep exposed to prolonged cold environments had elevations in both plasma T_3 and T_4. Some studies done on laboratory animals seem to favour a very minor participation of T_4 in cold resistance (Le Blanc 1971), with a more important role for T_3 in thermogenesis during cold stress. Elevated thyroid hormone levels may increase the thermogenic capacity of skeletal muscle by influencing mitochondrial structure and membrane sodium-potasium ATPase activity (Sasaki and Weekes 1986). T_3 plasma concentration was significantly lower at 28°C than at -10°C (127.3 vs 185.0 ng $100ml^{-1}$) indicating that heat stress inhibited thyroid activity. Yousef et al. (1968) showed that even when cattle are force-fed via a rumen fistula to equalize food intake heat stress induced a reduction in the secretory rate of thyroid hormones. Anderson et al. (1962) suggested that the inhibitory response to heat was mediated through the central nervous system. The involvement of the hypothalamus in the control of hormonal thermal-defence mechanisms was discussed in detail by Gale (1973) and Anderson et al. (1962) demonstrated that warming of this

region of the brain inhibited the activation of the sympatheticoadrenomedullary system and inhibited thyroid activation which occurs normally during cold stress.

The observed changes in metabolic rate in response to temperature were not a result of changes in feed intake. The steers showed a reduction in visible shivering and the postural behavioral responses characterized by convex curve of the spine was less frequent, after the second week of acclimation to -10°C in agreement with the work of Young (1975). After 2 weeks exposure to cold, the elevated heat production in the absence of shivering could be evidence that the animals had adapted metabolically to -10°C. During prolonged thermal exposure shivering intensity decreased in cattle (Young 1975) and in sheep (Schaefer et al. 1982) and therefore, shivering thermogenesis may not be the only cause for the increase heat production in steers exposed to -10°C. The metabolic heat production response to thermal exposure was slightly curvelinear in partial agreement with the report of Holmes and Close (1977). From our study it appears that elevated heat production in the cold acclimated steers could be attributed in part to non-shivering thermogenesis (NST), better known as the mechanism for producing heat by means other than shivering the function of which is to maintain homeostasis in a cold environment (Webster 1974).

The absence of visible shivering does not totally rule out the possibility that mild shivering may have continued to contribute to heat production. Although no recordings of EMG were made, the posture of the steer (arched back) suggests that increased muscle tone is

maintained in the cold. On exposure to cold, one organ recognized as being involved in the maintenance of body temperature is brown adipose tissue (BAT) (Smith 1961, Smith 1964). Although, adult ruminants are thought to lack BAT, recent studies on laboratory animals suggest that white adipose tissue (WAT) after prolonged exposure to cold is structurally similar to BAT (Loncar et al 1986, 1989). The involvement of the transformed WAT in heat production has not been investigated. Nevertheless, heat production in BAT is triggered by noradrenaline (NA) released from the sympathetic nerves innervating individual adipocytes.

Chronic exposure to cold induces increased plasma concentration of adrenaline and NA in sheep (Christopherson et al. 1978) and their stimulatory effect on lipolysis is known (Webster 1974). NA binding to adrenergic receptors on BAT adipocytes initiates a series of biochemical events that result in increased oxygen consumption and thus heat production (Nedergaard et al. 1982).

The possible mechanism involved has been described recently by Yoshiaki (1989) who reported that in rats exposed to cold, cAMP concentration in both BAT and WAT adipose tissues showed a stepwise decrease and that there was significant correlation between lipolysis and plasma cAMP response suggesting an involvement of the adenylate cyclase-cAMP system in NST via lipid metabolism. It is possible that organs other than BAT are responsible for NST in mature ruminants.

The multiplicity of sites of thermoregulatory heat production could be generally summarized by the following statement of Jansky (1973): "NST occurs in new-born mammals and in those that hibernate.

In some adults it can be induced by adaptation to cold. In small mammals, NST produces approximately the same amount of heat as shivering. It may become less important with increasing body weight of the animals. NST is localized mainly in skeletal muscles and brown adipose tissue. Small amounts of NST may come from liver, intestine, heart and brain".

Since no feed refusals occured in this study, the depressed metabolic rate observed in steers exposed to 28°C could have been caused by other physiological thermoregulatory responses mediated most likely by the hypothalamus. Heat may induce a depression of the anabolic and catabolic hormonal secretions most likely via the anterior pituitary (Anderson 1962, Gale 1973) resulting in the slowing down of the metabolic processes (Webster 1976).

There was a low but significant positive correlation between the heart rate ($r^2 = 0.53$), T_3 ($r^2 = 0.41$) and resting frequency of reticular contractions which may suggest that the resting frequency of reticular contraction increases with a rise in heart rate and T_3 plasma concentration. This may indicate that the resting frequency of reticular contraction, heart rate and plasma T_3 could be elevated during chronic cold exposure. The activation of the HPA and SA systems (see literature review) by stressful conditions is believed to influence target organs such as the heart, thyroid gland and the digestive system including the forestomachs of the ruminants. In this study prolonged thermal stress had a significant effect on the duration of reticular contractions and the frequency of reticular contractions which showed a quadratic response ($P<0.1$) during

resting. The decrease in the duration of reticular contractions at rest in the cold-acclimated steers and the slight increase in the resting frequency of contractions during prolonged exposure to -10°C indicates that low temperature has an effect on form and pattern of reticular motility, which was independent of feed intake because feed intake was maintained constant during this experiment.

The lack of significant effect of temperature on rumen movements is in partial agreement with the studies of Lirette et al. (1988) which showed substantial changes in frequency of ruminal contractions of steers exposed to an acute cold stress but a small and non significant change in reticulum contraction frequency. Westra and Christopherson (1976) reported a significant increase in the frequency of reticular contractions in sheep subjected to cold treatment. However, they used closely shorn sheep which would have been more responsive to cold because of reduced insulation.

Exposure of steers to -10°C in this study may not have constituted a sufficient severity of cold to evoke a convincingly measurable physiological digestive response in frequency and amplitude of rumen and reticular contractions. Large mammals are able to withstand colder temperatures than smaller animals because of their more favourable surface-to-mass ratio coupled with the ability to develop an effective thermal insulation and behavioural thermoregulation (Hardy et al. 1970, Christopherson and Young 1986). Our results cannot be fully reconciled with those of Attebery and Johnson (1969) because of differences in temperature treatments and

the duration of exposure used. This study is the first to report on effect of prolonged thermal exposure on duration of reticular contractions of steers during resting and may indicate that cold exposure not only increases frequency but also reduces duration of reticular contractions and this is consistent with the proposal of Leek and Harding (1975) who suggested that the activity of the RR could be a function of of both pattern and form of RR contractions. The duration and amplitude of ruminal contractions were not measured because, unlike the reticulum, where the characteristic biphasic/triphasic contractions confirmed the location of the catheters, the position of the catheters in the rumen is difficult to predict from the ruminal contractions.

The absence of marked differences in the effect of prolonged thermal exposure on the frequency, duration and amplitude of reticular contractions during rumination and feeding might also be attributed to behavioural and postural responses to stressful environments. Farm animals may seek to maximize their sensory pleasure and thus optimize their behavioural responses to stressful and unfriendly environments (Cabanac 1987).

The tacit or rather explicit assumption that the contractions of the forestomachs are important in regulating the flow of material from the rumen to the abomasum (Titchen 1968) is very much appreciated and it may be added that these contractions in concert with the well known peristaltic movements of the digestive system may provide a propulsive thrust to the movement of the already processed digesta. From this study, we noted that motility of the reticulo-rumen may not be the major limiting factor in so far as the

apparent digestibility of dietary constituents is concerned. This is discussed below.

The changes in apparent digestibility of dietary constituents were not a result of differences in feed intake because feed consumption was maintained constant. The changes in OM, DM, and NDF whose major site of degradation is thought to be the rumen (Kelly and Christopherson 1989) appear to be associated with changes in the rumen fluid rate constants in the present study. The significant negative correlation between digestibility of OM, DM, NDF and CP and FOR indicates that as the dilution of the rumen content increases, the digestibility will decrease implying that at higher dilution rates the escape of undigested material from the RR is enhanced.

It is proposed that rumen fluid dynamics contributed to depressed dietary constituent digestibilities in steers exposed to 10°C. The changes in N digestibility for steers varied amongst treatments and the digestibilities at -10°C and 28°C were similar. However, at the intermediate temperature (10°C) the digestibility of feed N was significantly depressed and there was a close relationship between N digestibility and FRC indicative of the direct influence of the FRC on the feed N. Our suggestion that FRC influences feed protein degradation is consistent with the few <u>in vivo</u> studies so far conducted (Walker et al. 1975) and reviewed (Bull et al. 1979) which indicates that rumen fluid outflow rate significantly affects N degradation and enhances rumen microbial synthesis.

Increased fluid outflow rate at 10°C potentially reduced time for fiber digestion which resulted in reduced total tract digestibility of DM, OM and NDF. Similarly, digestibility of feed N

was influenced by the fluid outflow rate which in addition, may increase bypass of dietary protein flowing with the liquid (Owens et al. 1977).

The changes in FRC and the duration of reticular contractions in the present study, frequency of reticular contractions (Westra and Christopherson 1976, Kennedy 1985), frequency and amplitude (Attebery and Johnson 1969), reduced particulate and fluid retention time in the RR (Westra and Christopherson 1976, Kennedy et al. 1976, 1978, Christopherson and Kennedy 1983) and in the whole digestive tract (Warren et al. 1974) have been proposed as possible mechanisms resulting in changes in digestibilities in ruminants exposed to different thermal environments. The overall control mechanism is regulated by the central and peripheral nervous systems modulated probably by the the release of the endogeneous putative and well established neurotransmiters (see literature review).

Although the PRCs were not significantly affected by prolonged thermal treatment in this study, there was a slight non-significant increase at -10°C and a general decrease towards the higher temperature of 28°C. The range of the PRC values is within the limits determined at thermoneutral temperatures by other researchers (Ehle et al. 1984, Pond et al. 1987, 1989). The increase of PRC at a lower temperature exposure might be a result of the small increase in frequency of reticular contractions during resting. On the other hand, the reduced duration of reticular contractions might limit the time that the reticulo-omasal orifice is open. This would be expected to reduce outflow of digesta.

Recently, Okine and Mathison (1989b) have shown that digesta turnover is closely related to duration of reticular contractions. Okine et al. loaded the rumen with weights and hence might have created an unphysiological condition which could be difficult to compare with our results. Nevertheless, in this regard the reduced duration of contraction in the cold is paradoxical, since it was associated with the highest particulate rate constant. Kennedy calculated that in sheep the quantity of digesta flowing per contraction of the reticulum was reduced in the cold. This observation is consistent with the reduced duration of contraction. The small nonsignificant increase in RDMC at 28°C was associated with a low FRC suggesting that rumen fluid dynamics might influence particulate kinetics.

The quadratic response of FRC with prolonged thermal exposure, low rumen volume (RV) at 28°C and the significant changes in water intake, indicates an influence of environmental temperature on rumen fluid dynamics which could be induced by the normal thermoregulatory water balance requirement. The rumen fluid responses of cattle in the present experiment differ somewhat from results with sheep (see Table 10). In experiments in which water was directly infused into the rumen it was demonstrated that changes in blood parameters induced by restricted water intake were restored to normal (Bianca 1970) suggesting that absorption of water from the rumen was rapid. From the present study, the slightly lower RV and lower FRC at 28°C may be a result of a relatively higher rate of water absorption from the rumen.

It is generally accepted that thermal stress significantly

affects intra and extravascular blood parameters (Horowitz et al. 1989, Sodhi et al. 1975, Jain 1986). At higher environmental temperatures, excessive cutaneous and respiratory water loss results in the reduction of blood volume which in turn stimulates the body's homeostatic control mechanism whose priority is to maintain a constant blood volume despite the fluctuations in water balance (Jain 1986). To achieve this state of constancy mobilization of water from various water pools including the rumen is necessary.

Taken together, the faster flushing of feed N at 10°C and thus bypassing the microbial upgrading processing stage in the RR, may not be beneficial to the host especially when poor quality feed is offered. Judging from the apparent digestibility of the various dietary constituents, it appears that the ability to utilize feed efficiently at an intermediate, but comfortable temperature (10°C) is lower compared to -10°C and 28°C. In contrast, at -10°C the energy requirement of the steers, as evident from the increased heat production, was much higher. Under normal circumstances animals would increase voluntary feed intake to offset the energy deficit (Christopherson 1976) but this possibility was limited in this study by maintaining a constant feed intake. With the increased energy costs at -10°C the extraction of energy from the roughage offered was elevated, though not as high as at 28°C. The increased apparent digestibility at both -10 and 28°C was associated with reduced FRC.

The digestibility patterns in this study and that of Blaxter and Wainman (1961) contrary to results based on several studies with sheep, suggests a non-linear digestive response to prolonged thermal exposure.

CONCLUSIONS

The major conclusions from this study are outlined below.

1. Water intake was significantly ($P<0.05$) lower at -10°C while the steers consumed more water at 28°C.
2. The so-called stress "indicators" heart rate, respiratory rate and rectal temperature were significantly ($P<0.05$) affected by prolonged thermal exposure suggesting that the animals were stressed at -10 and 28°C.
3. The thermal response in metabolic heat production was significant ($P<0.05$) and slightly curvilinear with the highest value at -10°C and lowest at 28°C. (8.24 vs 4.90 $WKg^{-3/4}$)
4. Plasma triiodothyronine but not thyroxine concentration was significantly ($P<0.05$) elevated at -10°C compared to 10 and 28°C.
5. The duration of reticular contraction during resting was significantly ($P<0.05$) reduced by -10°C exposure and the frequency showed a quadratic response ($P<0.1$) with prolonged thermal treatment.
6. The rumen fluid rate constant showed a quadratic response ($P<0.1$) with thermal exposure but the particulate passage rate constant estimated from chromium mordanted fiber was unaffected.
7. The changes in apparent digestibility of dry matter, organic matter, neutral detergent fiber and crude protein were significant ($P>0.05$) and non-linear with the lowest values at 10°C, intermediate at 10°C and highest at 28°C.

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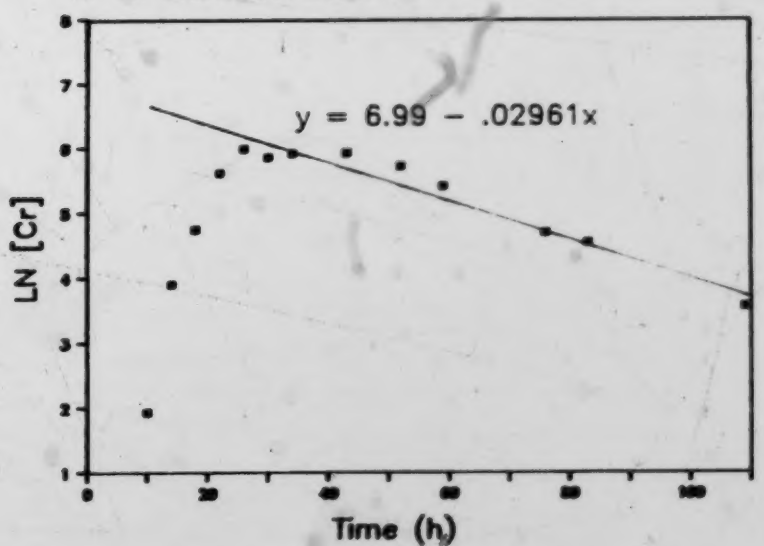
Appendix Table 1. Comparison between particulate rate constants based on ruminal and fecal sampling. (Lsmeans±S.E.M*, h^{-1}).

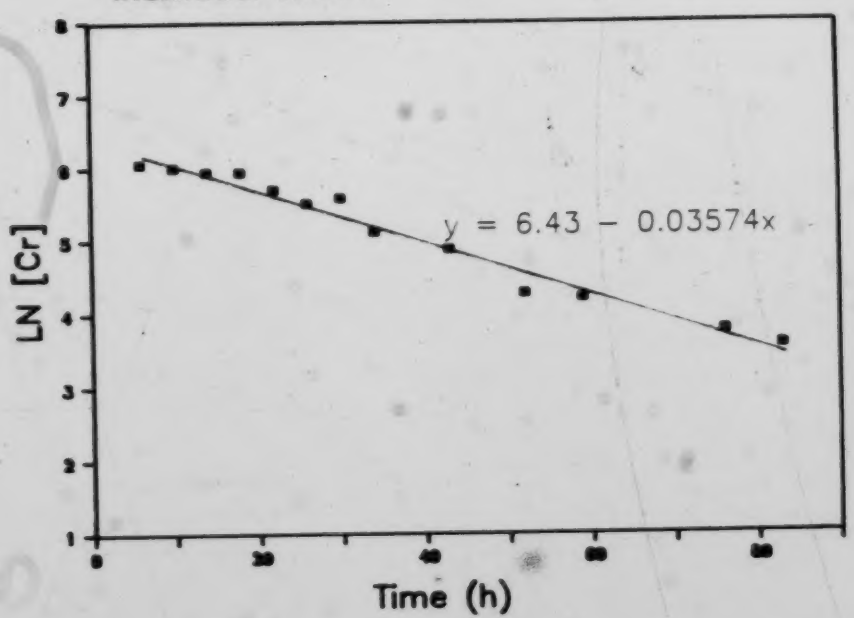
	Thermal treatment (°C)			
	-10	10	28	S.E.M
Ruminal site	4.2	3.9	3.3	0.3
Fecal Site	4.6	3.7	3.7	0.4

*S.E.M of treatment means (n 6)

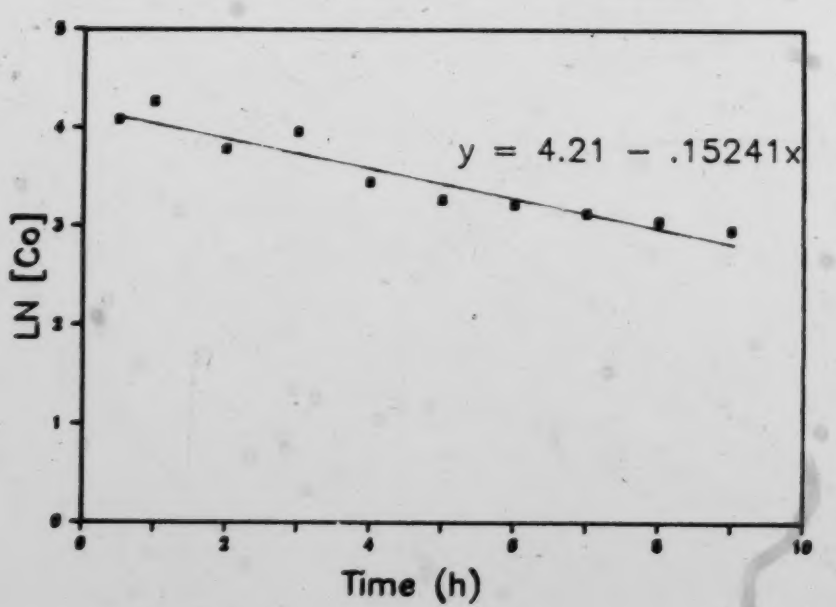
Appendix Figure 1. Example of marker–concentration curve of chromium–mordanted hay in the faeces of an individual steer.

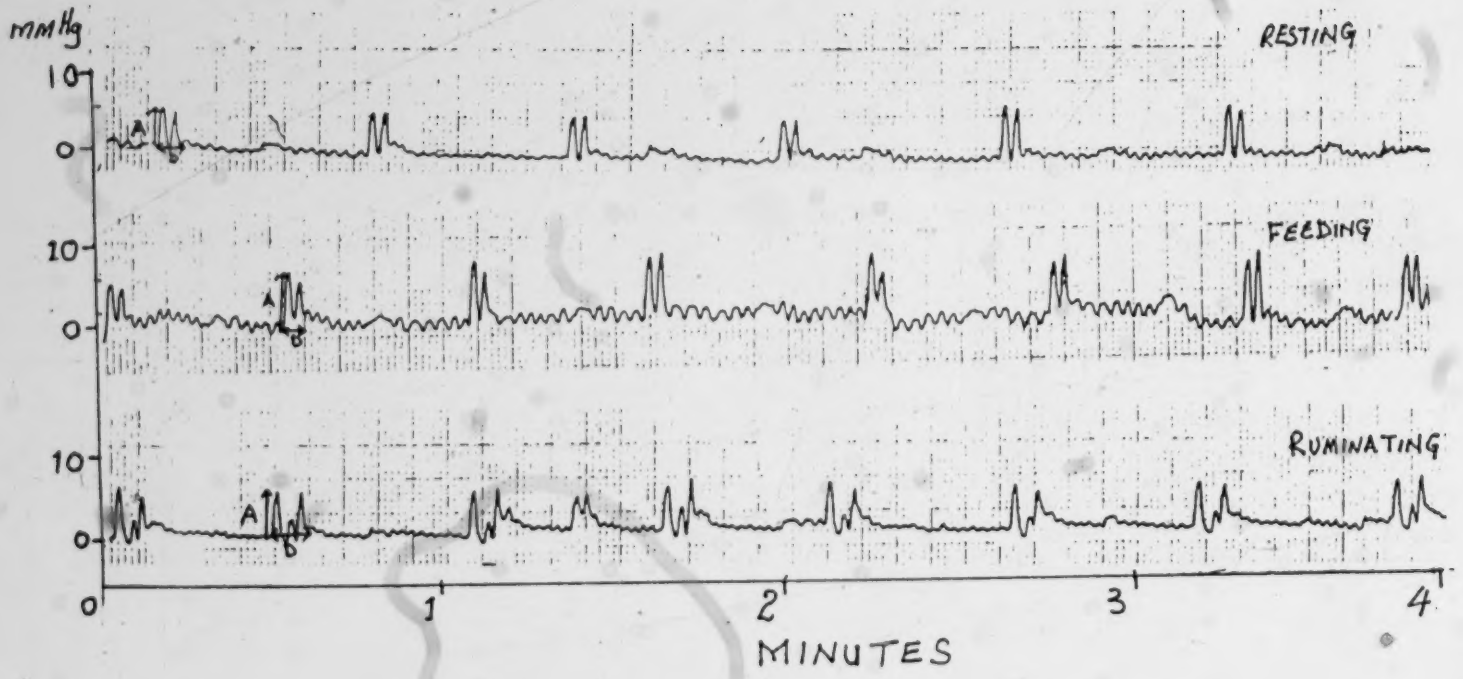


Appendix Figure 2. Example of a marker–concentration curve of chromium–mordanted hay in the rumen of an individual steer.



Appendix Figure 3. Example of a marker–concentration curve of CoEDTA in the rumen of an individual steer.





Appendix Figure 4. An example of a typical pressure peak recording of the reticular contractions of an individual steer during resting, feeding and ruminating. Notice A - Amplitude and D - Duration.